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(FILE 'HOME' ENTERED AT 16:52:00 ON 19 MAY 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH' ENTERED AT 16:52:15 ON 19 MAY 2003

L1 102 S E4ORF4
L2 771460 S VECTOR OR PLASMID
L3 15 S L1 AND L2
L4 9 DUP REM L3 (6 DUPLICATES REMOVED)

=> d bib ab 1-9 l4

L4 ANSWER 1 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2003:55096 BIOSIS
DN PREV200300055096
TI Transgene expression systems.
AU Kaplan, Johanne; Armentano, Donna; Gregory, Richard J.
ASSIGNEE: Genzyme Corporation
PI US 6485720 November 26, 2002
SO Official Gazette of the United States Patent and Trademark Office Patents, (Nov. 26 2002) Vol. 1264, No. 4, pp. No Pagination.
http://www.uspto.gov/web/menu/patdata.html. e-file.
ISSN: 0098-1133.
DT Patent
LA English
AB The present invention relates to transgene expression systems, related compositions comprising the transgene expression systems, and methods of making and using them. Preferred systems employ an adenovirus transgene expression **vector** comprising DNA encoding a transgene which codes for a desired product operably linked to expression control sequence, and at least a portion of the adenovirus E3 region and certain portions of the E4 region. The E4 portions comprise the open reading frame sequence known as E4ORF3 and at least one other portion of E4. Preferably the E4 portion of the **vector** (or "E4 cassette") includes E4ORF3 and at least one other portion selected from **E4ORF4**, E4ORF6/7 and E4ORF3/4. The invention has a number of important features including improving persistency of transgene expression in a desired host cell. The transgene expression systems of the present invention are useful for a variety of applications including providing persistent cellular expression of the transgene in vitro and in vivo.

L4 ANSWER 2 OF 9 MEDLINE
AN 2002099859 MEDLINE
DN 21819281 PubMed ID: 11829524
TI The nonapoptotic pathway mediating thymidine kinase/ganciclovir toxicity is reduced by signal from adenovirus type 5 early region 4.
AU Katabi Maha; Yuan Shala; Chan Helen; Galipeau Jacques; Batist Gerald
CS McGill Center for Translational Research in Cancer, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Quebec, H3T 1E2, Canada.
SO MOLECULAR THERAPY, (2002 Feb) 5 (2) 170-6.
Journal code: 100890581. ISSN: 1525-0016.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200209
ED Entered STN: 20020207
Last Updated on STN: 20020926
Entered Medline: 20020925
AB Suicide gene therapy using thymidine kinase/ganciclovir (Tk/GCV) yields highly variable results, in vitro and in vivo. To determine the reasons for such variations, we examined cellular mechanisms mediating its

cytotoxicity in view of their interaction with adenoviral **vectors** (Ad) used for gene delivery. Here we report that the presence of adenovirus early region 4 (AdE4)-encoded viral proteins significantly decreases toxicity of Tk/GCV. The E4 region-encoded proteins exerted this effect when found on the adenoviral delivery **vector** and when provided in trans in Tk retrovirally transduced cells. The apoptotic response was assessed in GCV-treated cells. The decrease in toxicity caused by AdE4 proteins was not correlated with apoptotic response, as measured by internucleosomal DNA degradation and TUNEL assays. Our results indicate that apoptosis is not the only mechanism of Tk/GCV-induced cell death and that other mechanisms equally important in determining the success of such a gene therapy strategy should be considered when optimizing treatment conditions.

L4 ANSWER 3 OF 9 MEDLINE
AN 2001491668 MEDLINE
DN 21427508 PubMed ID: 11536041
TI Toxicity of human adenovirus **E4orf4** protein in Saccharomyces cerevisiae results from interactions with the Cdc55 regulatory B subunit of PP2A.
AU Roopchand D E; Lee J M; Shahinian S; Paquette D; Bussey H; Branton P E
CS Department of Biochemistry, McGill University, McIntyre Medical Building, Montreal, Quebec, Canada, H3G 1Y6.
SO ONCOGENE, (2001 Aug 30) 20 (38) 5279-90.
Journal code: 8711562. ISSN: 0950-9232.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200109
ED Entered STN: 20010906
Last Updated on STN: 20030227
Entered Medline: 20010927
AB The **E4orf4** protein of human adenovirus induces p53-independent apoptosis, a process that may promote cell death and viral spread. When expressed alone, **E4orf4** kills transformed cells but not normal human cells. The only clear target of **E4orf4** in mammalian cells is the Balpha (B55) subunit of protein phosphatase 2A (PP2A), a member of one of three classes of regulatory B subunits. Here we report the effects of **E4orf4** in Saccharomyces cerevisiae, which encodes two PP2A regulatory B subunits, CDC55 and RTS1, that share homology with mammalian B and B' subunits, respectively. **E4orf4** expression was found to be toxic in yeast, resulting in the accumulation of cells in G2/M phase that failed to grow upon removal of **E4orf4**. **E4orf4** -expressing yeast also displayed an elongated cell morphology similar to cdc55 deletion strains. **E4orf4** required CDC55 to elicit its effect, whereas RTS1 was dispensable. The recruitment of the PP2A holoenzyme by **E4orf4** was entirely dependent on Cdc55. These studies indicate that **E4orf4**-induced apoptosis in mammalian cells and cell death in yeast require functional interactions with B-type subunits of PP2A. However, some inhibition of growth by **E4orf4** was observed in the cdc55 strain and with an **E4orf4** mutant that fails to interact with Cdc55, indicating that **E4orf4** may possess a second Cdc55-independent function affecting cell growth.

L4 ANSWER 4 OF 9 MEDLINE
AN 2001092668 MEDLINE
DN 20578214 PubMed ID: 11134292
TI Caspase activation by adenovirus **e4orf4** protein is cell line specific and Is mediated by the death receptor pathway.
AU Livne A; Shtrichman R; Kleinberger T
CS The Gonda Center of Molecular Microbiology, The Bruce Rappaport Faculty of Medicine, Technion, Haifa 31096, Israel.
SO JOURNAL OF VIROLOGY, (2001 Jan) 75 (2) 789-98.

Journal code: 0113724. ISSN: 0022-538X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200101
ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010125
AB Adenovirus **E4orf4** protein has been shown to induce transformed cell-specific, protein phosphatase 2A-dependent, and p53-independent apoptosis. It has been further reported that the **E4orf4** apoptotic pathway is caspase-independent in CHO cells. Here, we show that **E4orf4** induces caspase activation in the human cell lines H1299 and 293T. Caspase activation is required for apoptosis in 293T cells, but not in H1299 cells. Dominant negative mutants of caspase-8 and the death receptor adapter protein FADD/MORT1 inhibit **E4orf4**-induced apoptosis in 293T cells, suggesting that **E4orf4** activates the death receptor pathway. Cytochrome c is released into the cytosol in **E4orf4**-expressing cells, but caspase-9 is not required for induction of apoptosis. Furthermore, **E4orf4** induces accumulation of reactive oxygen species (ROS) in a caspase-8- and FADD/MORT1-dependent manner, and inhibition of ROS generation by 4,5-dihydroxy-1, 3-benzene-disulfonic acid (Tiron) inhibits **E4orf4** -induced apoptosis. Thus, our results demonstrate that **E4orf4** engages the death receptor pathway to generate at least part of the molecular events required for **E4orf4**-induced apoptosis.

L4 ANSWER 5 OF 9 MEDLINE
AN 2002150616 MEDLINE
DN 21880845 PubMed ID: 11883000
TI Mutational analysis of early region 4 of bovine adenovirus type 3.
AU Baxi M K; Robertson J; Babiuk L A; Tikoo S K
CS Virology Group, Veterinary Infectious Diseases Organization, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5E3.
SO VIROLOGY, (2001 Nov 10) 290 (1) 153-63.
Journal code: 0110674. ISSN: 0042-6822.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200204
ED Entered STN: 20020311
Last Updated on STN: 20020404
Entered Medline: 20020402
AB The primary objective of characterizing bovine adenovirus type 3 (BAV3) in greater detail is to develop it as a **vector** for gene therapy and vaccination of humans and animals. A series of BAV3 early region 4 (E4) deletion-mutant viruses, containing deletions in individual E4 open reading frames (Orf) or combinations of Orfs, were generated by transfecting primary fetal bovine retinal cells with E4-modified genomic DNA. Each of these mutants was further analyzed for growth kinetics, viral DNA accumulation, and early-late protein synthesis. Mutant viruses carrying deletions in Orf1, Orf2, Orf3, or Orf4 showed growth characteristics similar to those of the E3-deleted BAV3 (BAV302). DNA accumulation and early/late protein synthesis were also indistinguishable from those of BAV302. However, mutant viruses carrying a deletion in Orf5, Orfs 1-3 (BAV429), or Orfs 3-5 (BAV430) were modestly compromised in their ability to grow in bovine cells and express early/late proteins. E4 mutants containing larger deletions, Orfs 1-3 (BAV429) and Orfs 3-5 (BAV430), were further tested in a cotton rat model. Both mutants replicated as efficiently as BAV3 or BAV302 in the lungs of cotton rats. BAV3-specific IgA and IgG responses were detected in serum and at the mucosal surfaces in cotton rats inoculated with mutant viruses. In vitro

and in vivo characterization of these E4 mutants suggests that none of the individual E4 Orfs are essential for viral replication. Moreover, successful deletion of a 1.5-kb fragment in the BAV3 E4 region increased the available insertion capacity of replication-competent BAV3 **vector** (E3-E4 deleted) to approximately 4.5 kb and that of replication-defective BAV3 **vector** (E1a-E3-E4 deleted) to approximately 5.0 kb. This is extremely useful for the construction of BAV3 **vectors** that express multiple genes and/or regulatory elements for gene therapy and vaccination.

L4 ANSWER 6 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2001:185655 BIOSIS
DN PREV200100185655
TI Transgene expression systems.
AU Kaplan, Johanne (1); Armentano, Donna; Gregory, Richard J.
CS (1) Sherborn, MA USA
ASSIGNEE: Genzyme Corporation
PI US 6100086 August 08, 2000
SO Official Gazette of the United States Patent and Trademark Office Patents, (Aug. 8, 2000) Vol. 1237, No. 2, pp. No Pagination. e-file. ISSN: 0098-1133.
DT Patent
LA English
AB The present invention relates to transgene expression systems, related pharmaceutical compositions, and methods of making and using them. Preferred systems employ an adenovirus transgene expression **vector** comprising DNA sequence encoding a transgene which codes for a desired product, expressibly contained within an adenovirus **vector** containing at least a portion of the E3 region and certain portions of the E4 region. The E4 portions comprise the open reading frame sequence known as E4ORF3 and at least one other portion of E4. Preferably the E4 portion of the **vector** (or "E4 cassette") includes E4ORF3 and at least one other portion selected from **E4ORF4**, E4ORF6/7 and E4ORF3/4. The invention has a number of important features including improving persistency of transgene expression in a desired host cell. The transgene expression systems of the present invention are useful for a variety of applications including providing persistent cellular expression of the transgene in vitro and in vivo.

L4 ANSWER 7 OF 9 MEDLINE DUPLICATE 1
AN 1999099014 MEDLINE
DN 99099014 PubMed ID: 9882328
TI Analysis of synthesis, stability, phosphorylation, and interacting polypeptides of the 34-kilodalton product of open reading frame 6 of the early region 4 protein of human adenovirus type 5.
AU Boivin D; Morrison M R; Marcellus R C; Querido E; Branton P E
CS Departments of Biochemistry, McGill University, Montreal, Quebec, Canada H3G 1Y6.
SO JOURNAL OF VIROLOGY, (1999 Feb) 73 (2) 1245-53. Journal code: 0113724. ISSN: 0022-538X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199902
ED Entered STN: 19990301
Last Updated on STN: 19990301
Entered Medline: 19990218
AB The 34-kDa early-region 4 open reading frame 6 (E4orf6) product of human adenovirus type 5 forms complexes with both the cellular tumor suppressor p53 and the viral E1B 55-kDa protein (E1B-55kDa). E4orf6 can inhibit p53 transactivation activity, as can E1B-55kDa, and in combination these viral proteins cause the rapid turnover of p53. In addition, E4orf6-55kDa complexes play a critical role at later times in the regulation of viral

mRNA transport and shutoff of host cell protein synthesis. In the present study, we have further characterized some of the biological properties of E4orf6. Analysis of extracts from infected cells by Western blotting indicated that E4orf6, like E1A and E1B products, is present at high levels until very late times, suggesting that it is available to act throughout the infectious cycle. This pattern is similar to that of **E4orf4** but differs markedly from that of another E4 product, E4orf6/7, which is present only transiently. Synthesis of E4orf6 is maximal at early stages but ceases completely with the onset of shutoff of host protein synthesis; however, it was found that unlike E4orf6/7, E4orf6 is very stable, thus allowing high levels to be maintained even at late times. E4orf6 was shown to be phosphorylated at low levels. Coimmunoprecipitation studies in cells lacking p53 indicated that E4orf6 interacts with a number of other proteins. Five of these were shown to be viral or virally induced proteins ranging in size from 102 to 27 kDa, including E1B-55kDa. One such species, of 72 kDa, was shown not to represent the E2 DNA-binding protein and thus remains to be identified. Another appeared to be the L4 100-kDa nonstructural adenovirus late product, but it appeared to be present nonspecifically and not as part of an E4orf6 complex. Apart from p53, three additional cellular proteins, of 84, 19, and 14 kDa were detected by using an adenovirus **vector** that expresses only E4orf6. The 19-kDa species and a 16-kDa cellular protein were also shown to interact with E4orf6/7. It is possible that complex formation with these viral and cellular proteins plays a role in one or more of the biological activities associated with E4orf6 and E4orf6/7.

L4 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2003 ACS
AN 1998:712374 CAPLUS
DN 129:311713
TI Adenoviral **vectors** comprising a modified e4 region but retaining e4orf3
IN Kaplan, Johanne; Armentano, Donna; Gregory, Richard J.
PA Genzyme Corp., USA
SO PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9846779	A1	19981022	WO 1998-US7839	19980414
	W: AU, CA, JP, US				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	US 6100086	A	20000808	US 1997-839679	19970414
	AU 9871335	A1	19981111	AU 1998-71335	19980414
	AU 727992	B2	20010104		
	EP 975785	A1	20000202	EP 1998-918408	19980414
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 2001524822	T2	20011204	JP 1998-544341	19980414
	US 6358507	B1	20020319	US 1999-416673	19991012
	US 2002028194	A1	20020307	US 2001-924925	20010808
	US 6485720	B2	20021126		
PRAI	US 1997-839679	A	19970414		
	WO 1998-US7839	W	19980414		
	US 1999-416673	A1	19991012		

AB Methods for making transgene expression systems are described. Preferred systems employ an adenovirus transgene expression **vector** comprising DNA encoding a transgene which codes for a desired product operably linked to expression control sequence, and at least a portion of the adenovirus E3 region and certain portions of the E4 region. The E4 portions comprise the open reading frame sequence known as E4ORF3 and at

least one other portion of E4. Preferably the E4 portion of the **vector** (or "E4 cassette") includes E4ORF3 and at least one other portion selected from **E4ORF4**, E4ORF6/7 and E4ORF3/4. The invention has a no. of important features including improving persistency of transgene expression in a desired host cell. These transgene expression systems are useful for a variety of applications including providing persistent cellular expression of the transgene in vitro and in vivo. Specifically, the transgene encoding CFTR was shown to be effectively expressed in lung airway epithelium. This expression system minimizes cell-mediated immune reactions against adenovirus **vectors** by use of E3 region encoding gp19K glycoprotein which adversely affects MHCI receptor. This system is therefore attractive for situations where repeat administration of the transgene may be required such as in gene therapy treatments requiring high or multiple dose protocols.

RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 9 OF 9 MEDLINE DUPLICATE 2
AN 1998362117 MEDLINE
DN 98362117 PubMed ID: 9696808
TI The early region 4 orf4 protein of human adenovirus type 5 induces p53-independent cell death by apoptosis.
AU Marcellus R C; Lavoie J N; Boivin D; Shore G C; Ketner G; Branton P E
CS Departments of Biochemistry, McGill University, Montreal, Quebec, Canada H3G 1Y6.
SO JOURNAL OF VIROLOGY, (1998 Sep) 72 (9) 7144-53.
Journal code: 0113724. ISSN: 0022-538X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199809
ED Entered STN: 19980925
Last Updated on STN: 19980925
Entered Medline: 19980916
AB Previous studies by our group showed that infection of human and rodent cells by human adenovirus type 5 (Ad5) results in the induction of p53-independent apoptosis and cell death that are dependent upon transactivation of early region 4 (E4). To identify which E4 products are involved, studies were conducted with p53-deficient human SAOS-2 cells infected with various Ad5 E4 mutants. An E4orf6-deficient mutant was defective in cell killing, whereas another that expressed only E4orf6 and **E4orf4** killed like wild-type virus, suggesting that E4orf6 may be responsible for cytotoxicity; however, a mutant expressing only **E4orf4** induced high levels of cell death, indicating that this E4 product may also be able to induce cytotoxicity. To define the E4 cell death-inducing functions more precisely, cDNAs encoding individual E4 products were introduced into cells by DNA transfection in the absence of other Ad5 proteins. In cotransfections with a cDNA encoding firefly luciferase, enzymatic activity was high in all cases except with **E4orf4**, where luciferase levels were less than 20% of those in controls. In addition, drug selection of several cell types following transfection with retroviral **vector** DNA encoding individual E4 products as well as puromycin resistance yielded a large number of cell colonies except when **E4orf4** was expressed. These data demonstrated that **E4orf4** is the only E4 product capable of independent cell killing. Cell death induced by **E4orf4** was due to apoptosis, as evidenced by 4',6-diamidino-2-phenylindole (DAPI) staining of cell nuclei in **E4orf4**-expressing cells. Thus, although E4orf6 may play some role, these results suggested that **E4orf4** may be the major E4 product responsible for induction of p53-independent apoptosis.